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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12Q 1/68, C07H 21/04, 21/02 — A1

(11) International Publication Number: WO 94/28174

(43) International Publication Date: 8 December 1994 (08.12.94)

(21) International Application Number: PCT/US94/05821

(22) International Filing Date: 23 May 1994 (23.05.94)

(30) Priority Data:
08/066,373 24 May 1993 (24.05.93) US

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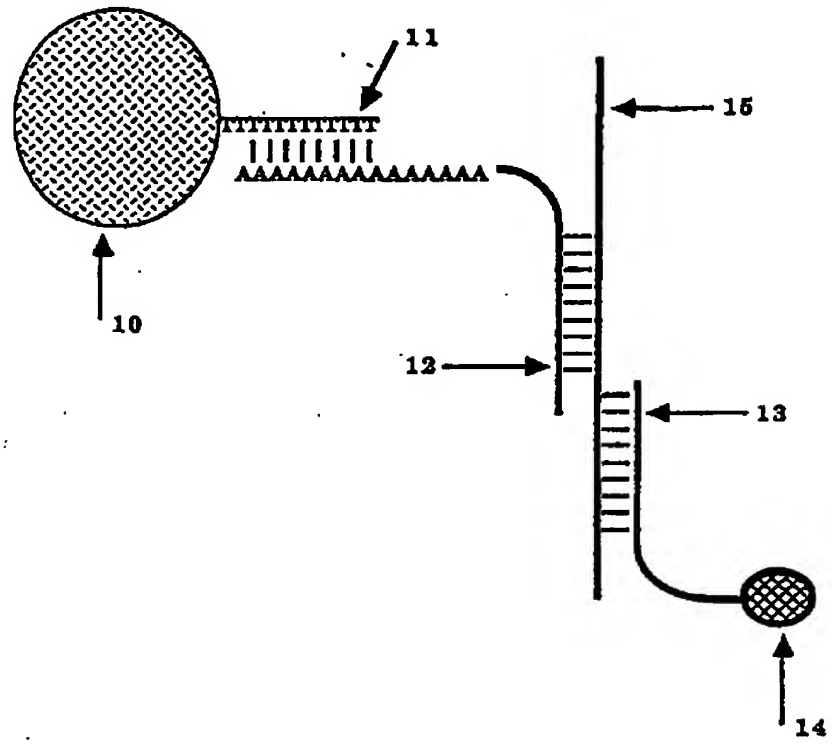
(81) Designated States: JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: NUCLEIC ACID PROBES FOR BACTERIA OF THE GENUS LEGIONELLA

(57) Abstract

Nucleic acid sequences which hybridize preferentially to the 16S or 23S rRNA or rDNA of Legionella sp., L. pneumophila, L. Miodadei, an L. pneumophila, or a Legionella subset are taught. These organisms are the etiological agents of Legionaires' disease, Pontiac fever, Pittsburgh pneumonia and other infections. The nucleic acids are useful in the detection of these pathogenic microorganisms, such as in sandwich assays. Probes based on these sequences and kits containing the probes are also disclosed.



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NUCLEIC ACID PROBES FOR BACTERIA OF THE GENUS LEGIONELLA

This invention relates to nucleic acids, probes, kits, and methods for the detection of pathogenic organisms, including *Legionella sp.*, believed to be involved with Legionnaire's disease, Pontiac fever, Pittsburgh pneumonia, and several other diseases.

Background of the Invention

Legionella were first discovered in 1976 following an outbreak of 182 cases of pneumonic illness (termed Legionnaires' disease) occurred at a state convention of the American Legion in Philadelphia, Pennsylvania, which resulted in 29 deaths. What is now known as *L. pneumophila* serotype 1 was originally isolated by standard techniques for the isolation of rickettsiae and subsequently shown to be the etiological agent for Legion-naires' disease, a form of atypical pneumonia with other non-respiratory complications. Since that first outbreak, Legionellae have been implicated in Pontiac fever and Pittsburgh pneumonia. Pontiac fever is a non-pneumonic febrile self-limiting illness caused by *L. pneumophila*. Pittsburgh pneumonia is a pulmonary legionellosis caused by *L. micdadei*.

Legionellae are gram negative, aerobic, facultative intracellular, parasitic bacteria found to be practically ubiquitous in fresh water supplies (including evaporative condensers, cooling towers, and potable water). It is believed that Legionellae cause disease when contaminated water is inhaled, often leading to epidemic or clustered outbreaks. In addition to community-acquired cases, legionellae may be a major cause of nosocomial infections. Along with hospitalization, host risk factors include smoking, advanced age, chronic lung disease, and immunosuppression.

The family Legionellaceae contains the single genus *Legionella* which includes some 29 species, 21 serogroups and 5 tentatively named species. According to DNA homology studies, *L. micdadei* is the most distant relative of *L. pneumophila*, and there is some who argue that *L. micdadei* should be properly classified as a member of the genus *Tatlockia*.

L. pneumophila, the primary cause of Legionnaire's disease, is the most common human isolate. The presence of legionellae in human clinical samples always provides clinically relevant information, as the bacteria are not considered normal human microflora.

Legionellae are slow growing organisms which are difficult to culture. Thus, isolation of *L. pneumophila* by laboratories can be difficult and time-consuming. Given the serious nature of the diseases, and the need to prescribe correct antibiotics, it is highly desirable for a physician to make a rapid and accurate diagnosis of the presence of these organisms. Current methods of detection of Legionellae include (a) culture; (b) direct fluorescence antibody (DFA); (c) nucleic acid probes for culture confirmation; and (d) serology (IFA). Serology is currently the most sensitive and specific test method. It is limited however, in that antibodies may persist in an individual's serum for years after infection.

Certain probes which are based on *Legionella* rRNAs are disclosed in WO 88/03957 "Nucleic Acid Probes for Detection and/or Quantification of Non-Viral Organisms", published June 2, 1988, Applicant: Gen-Probe, Inventors: Hogan et al. This application however, discloses a mixture of three different probes which can be used to differentiate *Legionella* bacterial from non-*Legionella* bacteria. No probes suitable for solitary use are reported. It would be desirable to have probes which, when used singly or in pairs can be used in various diagnostic assays involving *Legionella*.

Description of the Invention

One aspect of this invention is to provide nucleic acids complementary to unique nucleic acid sequences within the ribosomal RNA (rRNA) of legionellae, and which can

be used either singly or in pairs. It is a further aspect of the invention to provide for probes which either (1) specifically discriminate between *L. pneumophila* and other Legionellas; (2) specifically discriminate between a group of bacteria comprised of *L. pneumophila* and other species of *Legionella* (a *Legionella* cluster) and other bacteria; (3) specifically discriminate between *L. micdadei* and other Legionellas; or (4) specifically discriminate between *Legionella* and other genera.

Bacterial ribosomes contain three distinct RNA molecules which, at least in *Escherichia coli* are referred to as 5S, 16S, and 23S rRNAs. In eukaryotic organisms, there are four distinct rRNA species, generally referred to as 5S, 18S, 28S and 5.8S. These names are historically related to the size of the RNA molecules, as determined by their sedimentation rate. In actuality, however, rRNA molecules vary substantially in size between organisms. This notwithstanding, 5S, 16S and 23S rRNA are art-recognized names referring to rRNA molecules in any bacteria, including the legionellae and this convention will be used herein.

The probes of the present invention target either the 16S or the 23S rRNA molecules of various organisms of the genus *Legionella*.

Description of the Figures

Figure 1 is a diagram of a sandwich assay.

As used throughout the application and claims, the term "probe" will refer to synthetic or biologically produced nucleic acids, between 10 and 250 base pairs in length, which by design or selection, contain specific nucleotide sequences that allow specific and preferential hybridization under predetermined conditions to target nucleic acid sequences, and optionally contain a moiety for detection or for enhancing assay performance. A minimum of ten nucleotides is generally necessary in order to statistically obtain specificity and form stable hybridization products, and a maximum of 250 nucleotides generally represents an upper limit for sequences in which reaction parameters can be adjusted to determine mismatched sequences and preferential hybridization. Therefore, in general, a preferred length of a probe will be between 10

and 250 nucleotides. Probes may optionally contain certain constituents that pertain to their proper or optimal functioning under certain assay conditions. For example, probes may be modified to improve their resistance to nuclease degradation (such as by end-capping), to carry detection ligands (such as fluorescein, 32P, biotin, etc.) or to facilitate their capture onto a solid support (e.g. poly-deoxyadenosine "tails").

"Preferential hybridization" or "hybridizing preferentially" means that hybridization with the intended target nucleic acid results in a hybridization reaction product which is more stable than any hybridization reaction products resulting from hybridization with a non-target nucleic acid under identical conditions. It is well within the skill of the ordinary artisan to compare stability of hybridization reaction products and evaluate which one is more stable, i.e. determine which one has bound "preferentially".

As used herein, the terms "homology" and "homologous to" are meant to refer to the degree of similarity between two or more nucleic acid sequences, and is not meant to imply any taxonomic relatedness between organisms. The degree of similarity is expressed as a percentage, i.e. 90% homology between two sequences will mean that 90% of the bases of the first sequence are identically matched to the bases of the second sequence.

"*Legionella* cluster" means at least two members of the genus *Legionella*. Probes which identify a *Legionella* cluster will typically hybridize to rRNA of a plurality of *Legionella* species tested, (although not all *Legionella*).

"*L. pneumophila* cluster" means at least two strains of the species *L. pneumophila*. Probes which identify a *L. pneumophila* cluster will typically hybridize to rRNA of a plurality of *L. pneumophila* strains tested (although not all *L. pneumophila*).

"Specific" means that a nucleotide sequence will hybridize to a predetermined target sequence and will not substantially hybridize to a non-target sequence.

"Specifically discriminate" means that a probe will substantially hybridize to a predetermined target sequence and will not substantially hybridize to a non-target sequence.

"Hybridization" is a process by which, under predetermined reaction conditions, two partially or completely complementary strands of nucleic acid are allowed to come together in an antiparallel fashion to form a double stranded nucleic acid with specific and stable hydrogen bonds, following explicit rules pertaining to which nucleic acids bases may pair with one another.

"Substantial hybridization" means that the amount of hybridization observed will be such that one observing the results would consider the result positive in a clinical setting. Data which is considered "background noise" is not substantial hybridization.

"Stringent hybridization conditions" means approximately 35°C to 65°C in a salt solution of approximately 0.9 molar NaCl. Stringency may also be governed by such reaction parameters as the concentration and type of ionic species present in the hybridization solution, the types and concentrations of denaturing agents present, and the temperature of hybridization. Generally as hybridization conditions become more stringent, longer probes are preferred if stable hybrids are to be formed. As a rule, the stringency of the conditions under which a hybridization is to take place will dictate certain characteristics of the preferred probes to be employed. Such relationships are well understood and can be readily manipulated by those skilled in the art.

"*Legionella* sp." refers to any member of the genus *Legionella*, regardless of the species.

In accordance with this invention, there are provided nucleic acids having approximately 10 to 250 nucleotides which hybridize preferentially to rRNA or rDNA of a target organism selected from the group consisting of 1) *L. pneumophila*, 2) *L. micdadei*, 3) a *Legionella* cluster, or 4) a *L. pneumophila* cluster, or 5) all species of the genus *Legionella*. Under those same hybridization conditions, the nucleic acids of this invention do not substantially hybridize to the rRNA or rDNA of non-target organisms, or the host or environmental matrix which may be present in test samples. Probes which specifically discriminate between *L. pneumophila* and other *Legionella* species are useful in the diagnosis of Legionnaires' disease or Pontiac fever. Probes which specifically

discriminate between *L. micdadei* and other *Legionella* species are useful in the diagnosis of Pittsburgh pneumonia. Probes which specifically hybridize to a *Legionella* cluster are useful in detecting the presence of one or more organisms which makes up the particular cluster of bacteria. Probes which specifically discriminate between members of the genus *Legionella* and non-*Legionella* bacteria are useful in detecting the presence or absence of one or organisms belonging to the genus *Legionella*. Probes which specifically hybridize to a *L. pneumophila* cluster are useful in determining which strains (serotypes) of *L. pheumophila* are present. Probes which are either complementary to or at least 90% homologous to at least ten consecutive nucleic acids of the aforementioned nucleotides also form another aspect of this invention.

One embodiment of the nucleic acids and probes of this invention are those which are complementary to, at least 90% homologous with, or hybridize preferentially with regions of 16S rRNA or rDNA of either 1) *L. pneumophila*, 2) *L. micdadei*, 3) a *Legionella* cluster, 4) a *L. pneumophila* cluster, or 5) all *Legionella* species. The regions of 16S rRNA of particular interest include those indicated below. The numbering of these regions is by reference to the numbering used for *E. coli* rRNA designations.

L. pneumophila 16S rRNA positions 60 to 110, 1105 to 1165, and 1250 to 1315;

L. micdadei 16S rRNA positions 60 to 110, and 815 to 875;

Legionella sp. 16S rRNA positions 205 to 255, 425 to 475, 715 to 765, and 845 to 895 (for *Legionella* cluster probes);

Legionella sp. 120 to 175, and 800 to 870 (for *Legionella* genus probes).

Another embodiment of this invention includes nucleic acids and probes which are complementary to, at least 90% homologous with, or hybridize preferentially with regions of 23S rRNA or rDNA of either 1) a *L. pneumophila* cluster, 2) *L. micdadei*, 3) a *Legionella* cluster, or 4) all *Legionella* species. The regions of 23S rRNA of particular interest include:

L. micdadei 23S rRNA positions 285 to 335, 1195 to 1245, 1485 to 1565, and 1705 to 1755.

Legionella sp. 23S rRNA positions 285 to 335, 1485 to 1560 and 1705 to 1755 (for *Legionella* cluster probes)

Legionella sp. 23S rRNA positions 1565 to 1615 and 2270 to 2310 (for *Legionella* genus probes).

Preferably the nucleic acid composition is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides within sequences selected from the group of sequences defined by the group of probes consisting of: 2701, 2703, 2704, 2705, 2697, 2690, 2698, 2695, 2696, 2693, 2708, 2699, 2924, 2926, 2930, 2932, 2956, 2958, 2963, 2968, 2928, 2957, 2927, 2929, 2954, 2955, and 2959. The sequences of these probes are presented below.

A further embodiment of this invention includes a kit for the detection of either 1) *L. pneumophila*, 2) *L. micdadei*, 3) a *Legionella* cluster, 4) a *L. pheumophila* cluster, or 5) any *Legionella* species. The kit comprises a set of nucleic acids comprising at least two nucleic acids. Each nucleic acid is 10 to 250 nucleotides in length and is of a different base sequence composition. Each nucleic acid is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 2701, 2703, 2704, 2705, 2697, 2690, 2698, 2695, 2696, 2693, 2708, 2699, 2924, 2926, 2930, 2932, 2956, 2958, 2963, 2968, 2928, 2957, 2927, 2929, 2954, 2955, and 2959. A set of nucleic acids particularly suited for detecting *Legionella* is a two-probe sandwich assay. The kit additionally comprises reagents, compositions, instructions, disposable hardware and suitable packaging to allow marketing in a convenient assembly.

A further embodiment of the present invention includes methods for the detection of the presence of 1) *L. pneumophila*, 2) *L. micdadei*, 3) a *Legionella* cluster, 4) a *L. pneumophila* cluster, or 5) any *Legionella* species. The method comprises the steps of contacting a sample suspected of containing a target with at least one nucleic acid. The nucleic acid has approximately 10 to 250 nucleotides which hybridize preferentially to 1) *L. pneumophila*, 2) *L. micdadei*, 3) a *Legionella* cluster, 4) a *L. pneumophila* cluster, or 5) any *Legionella* species rRNA or rDNA. The method includes the step of imposing

hybridization conditions on the sample such that the nucleic acid binds preferentially to the target rRNA or rDNA to form nucleic acid complexes and detecting the complexes as an indication of the presence of the target organism(s). Preferably, the nucleic acid of the present invention is at least 90% homologous to a sequence comprising any ten consecutive nucleotides selected from the group consisting of sequences defined by probes 2701, 2703, 2704, 2705, 2697, 2690, 2698, 2695, 2696, 2693, 2708, 2699, 2924, 2926, 2930, 2932, 2956, 2958, 2963, 2968, 2928, 2957, 2927, 2929, 2954, 2955, and 2959.

The probes of the present invention provide the basis for development of a nucleic acid hybridization assay for the specific detection of legionellosis or its etiological agent in environmental samples such as water samples and clinical samples such as sputum, throat swabs, blood, urine, cerebrospinal fluid, skin, biopsy, saliva, synovial fluid, bronchial wash, bronchial lavage, or other tissue or fluid samples from human patient or veterinary subjects. The probes of the present invention also form the basis for confirmation of the presence or absence of 1) *L. pneumophila*, 2) *L. micdadei*, 3) a *L. pneumophila* cluster, 4) a *L. pneumophila* cluster, or 5) all *Legionella* species.

The first step taken in the development of the probes of the present invention involved the identification of the regions of 16S or 23S rRNA which potentially could serve as target sites for specific nucleic acid probes with the desired sensitivity. This included discovering which probe target sites were unique to *L. pneumophila* and *L. micdadei*, and discovering probe sites which were common to *Legionella*.

To accomplish the above analysis, precise alignments of legionellae 16S and 23S rRNA sequences were developed. The essentially complete 16S and 23S rRNA sequences of both *L. pneumophila* and *L. micdadei* were determined using standard laboratory protocols. The rDNAs so obtained were cloned into plasmid vectors from products produced by enzymic amplification (such as that described in Weisburg, 1991, J. Bacteriol. 173:697-703, which is incorporated herein by reference). The *L. pneumophila* and *L. micdadei* sequences were aligned with homologous sequences of other *Legionella* and non-*Legionella* rRNA sequences.

Based on the determined 16S and 23S rRNA sequences of *L. pneumophila* and *L. micdadei*, various probes were designed, and synthesized. The specific behaviors of the probes are dependent to a significant extent on the assay format in which they are employed. Conversely, the assay format will dictate certain of the optimal features of the particular probes.

The discovery that a single probe or a pair of probes could be generated with the extraordinary inclusivity and exclusivity characteristics of the present invention with respect to 1) *L. pneumophila*, 2) *L. micdadei*, 3) a *Legionella* cluster, 4) a *L. pneumophila* cluster, or 5) any *Legionella* species without incurring undesirable levels of cross-reactivity was unpredictable and unexpected. Further, the finding that a single probe that has enough hybridization capability and selectivity for the target organism to be useful in a diagnostic assay was also unexpected.

A first group of preferred probes are able to differentiate between *L. pneumophila* and other *Legionella* species, and are useful in determining the presence of *L. pneumophila* in a sample. These probes hybridize preferentially only to *L. pneumophila*, and referred to as Probes 2704, 2705, 2708 and 2690. Probe 2957 gives somewhat weak hybridization signals, but is specific for *L. pneumophila*. Also given are two probes, 2929 and 2954 which only hybridize preferentially to some *L. pneumophila* strains tested, although they do not substantially hybridize to other organisms. These two probes, hereinafter called "*L. pneumophila* cluster" probes are useful in determining particular strains and/or serotypes.

L. pneumophila 16S rRNA Probes

L. pneumophila Probe 2704 (31mer 48% G+C) (SEQ ID NO:1)
5'-TCG CCA TCT GTC TAG CAA GCT AGA CAA TGC T-3'

L. pneumophila Probe 2705 (30mer 50% G+C) (SEQ ID NO:2)
5'-ACT TTT AAG GAT TTG CTC CAG GTC GCC CCT-3'

L. pneumophila Probe 2708 (31mer 45% G+C) (SEQ ID NO:3)
5'-ACT ACG ACC GAC TTT TAA GGA TTT GCT CCA G-3'

L. pneumophila Probe 2690 (36mer 50% G+C) (SEQ ID NO:4)
5'-TAG AGT CCC CAC CAT CAC ATG CTG GCA ACT AAG GAT-3'

L. pneumophila 23S rRNA Probe

L. pneumophila Probe 2957 (36mer 42% G+C) (SEQ ID NO:5)
5'-TCA ATG ACT TCT CTA TAC CAA AAG GGT CAG AAC CAC-3'

L. pneumophila cluster 23S rRNA Probes

L. pneumophila cluster Probe 2929 (31mer 42% G+C) (SEQ ID NO:6)
5'-CTC TAT CGC CAA CTT TCC CAA ATT GTT CTA C-3'

L. pneumophila cluster Probe 2954 (31mer 48% G+C) (SEQ ID NO:7)
5'-GCA CCT CAG AGT TAT GGA AAA CCG GAT TTG C-3'

A second group of preferred probes are able to differentiate between *L. micdadei* and substantially all other *Legionella* species, as these probes are able to bind preferentially to *L. micdadei*. They are useful in determining the presence of *L. micdadei* in a sample, and are designated Probes 2699, 2703, 2932, 2956, 2958, 2963, and 2968.

Probe 2703 hybridizes to *L. micdadei* and *L. dumoffi*, but not to any other *Legionella* species, and may be used to detect the preseance of either or both of these microorganisms.

L. micdadei 16S rRNA PROBES

L. micdadei Probe 2699 (34mer 41% G+C) (SEQ ID NO:8)
5'-TTC GTC ACT AAC CTC ATT CAT AAG GCC AAC AAC T-3'

L. micdadei 23S rRNA PROBES

L. micdadei Probe 2932 (31mer 48% G+C) (SEQ ID NO:9)
5'-CTG TAT CGT GGT ACT TCC CAG AAC CTT CTA C-3'

L. micdadei Probe 2956 (30mer 57% G+C) (SEQ ID NO:10)
5'-GCC CAC CTC TCA GTG AAC CTT CTT CAG CCT-3'

L. micdadei Probe 2958 (32mer 59% G+C) (SEQ ID NO:11)
5'-GCA CCT CAG CCT TAA CAA GGG GCC GGA TTT GC-3'

L. micdadei Probe 2963 (32mer 44% G+C) (SEQ ID NO:12)
5'-CCT CTT CAG CTC ATT AAG CAT GTC AAT TCA CC-3'

L. micdadei Probe 2968 (37mer 47% G+C) (SEQ ID NO:13)
5'-TCA ATG ACT TCT CCG CAC ACC GTA GTG TCA GAA CCA C-3'

L. micdadei and *L. dumoffi* 16S rRNA PROBE

L. micdadei and *L. dumoffi* Probe 2703 (31mer 52% G+C) (SEQ ID NO:14)
5'-TCG CCA CCC ATC TAG TAA ACT AGA CCG TGC T-3'

A third group of preferred probes hybridize preferentially with a cluster of *Legionella* species compared to other bacteria. These probes are useful in distinguishing between various *Legionella* bacteria and strains.

Probe 2697 hybridizes to all *L. pneumophila* and three other *Legionella* species.

Probe 2698 hybridizes preferentially with all *Legionella* except for *L. bozemanii*, where hybridization is weak. Some non-substantial hybridization was noted with *Enterobacter agglomerans* and *Coxiella burnetii*.

Probe 2693 hybridizes with all *Legionella* species except for *L. micdadei*; some hybridization with *Acholeplasma laidlawii* was noted, but this is believed to be the result of an error in experimental manipulation, so this observation is not considered to be determinative. This probe also hybridizes with *Pseudomonas aeruginosa;* minor amounts of hybridization were noted with *Actinobacillus actinomycetamcomitans* and *Alteromonas putrefaciens, Coxiella burnetii* and *Wolbachia persica*.

Probe 2928 hybridizes to all *Legionella* species, and to certain other bacterial species: *Hafnia alvei, Morganella morganii, Proteus mirabilis, Providencia alcalifaciens, Serratia marcescens, Yersinia enterocolitica, Y. pseudotuberculosis* and *Francisella tularensis*. Weak hybridization was noted with *Edwardsiella tarda* and *Enterobacter agglomerans*.

Probe 2927 hybridizes to all *Legionella* and *Acinetobacter calcoaceticus, Aeromonas sobria, Edwardsiella tarda, Vibrio parahaemolyticus, Yersinia enterocolitica,* and *Y. pseudotuberculosis*. Lesser hybridization is noted with *Hafnia alvei, Proteus mirabilis, Providencia alcalifaciens, Serratia marcescens,* and *Mycoplasma hominis*. Even less

hybridization is observed with *Plesiomonas shigelloides, Salmonella typhimurium,* and *Corynebacterium glutamicum.*

Probe 2955 hybridizes to all *Legionella* (but not well with *L. micdadei* and *L. bozemanii*); it also hybridizes to *Neisseriae gonorrhoeae* and to a much lesser extent, *N. meningitidis.*

Probe 2494 hybridizes preferentially with various *Legionella* species, but does not hybridize to non-*Legionella* species.

Probe 2959 hybridizes with a group of the *L. pneumophila* strains and also with *L. dumoffii* (and weakly with *L. gormanii.*)

Legionella Cluster 16S rRNA PROBES

Legionella Cluster Probe 2697 (33mer 48% G+C) (SEQ ID NO:15)
5'-TTT CCC CAA GTT GTC CCC CTC TTC AAG GCA TAT-3'

Legionella Cluster Probe 2698 (33mer 48% G+C) (SEQ ID NO:16)
5'-TCT TAA CCT ATC AAC CCT CCT CCC CAC TGA AAG-3'

Legionella Cluster Probe 2693 (30mer 60% G+C) (SEQ ID NO:17)
5'-AGG CGG TCA ACT TAT CGC GTT TGC TGC GCC-3'

Legionella Cluster 23S rRNA PROBES

Legionella Cluster Probe 2928 (31mer 51% G+C) (SEQ ID NO:18)
5'-TAA GAC CAA CTT TCG TTC CTG CTC GAG CCG T-3'

Legionella Cluster Probe 2927 (30mer 47% G+C) (SEQ ID NO:19)
5'-TCA GAC TCG ATT TCT CTA CGG CTC CCT TAT-3'

Legionella Cluster Probe 2955 (30mer 50% G+C) (SEQ ID NO:20)
5'-GCA CAC TTC TCA ATG CAC CTT CAT CAG CCT-3'

Legionella Cluster Probe 2924 (31mer 42% G+C) (SEQ ID NO:21)
5'-CAC AGT CAT CAT CAA AGT CCA GTG CAA AAC T-3'

Legionella Cluster Probe 2959 (32mer 50% G+C) (SEQ ID NO:22)
5'-CCT CTC CAG CTC TGA AAG TAA ATC CCA TCA CC-3'

A fourth group of preferred probes is able to differentiate between *Legionella* and non-*Legionella* species. They hybridize preferentially with all *Legionella* species and do not substantially hybridize with non-*Legionella* species. These are referred to as *Legionella* genus probes.

Probe 2696 hybridizes with all *Legionella* except (*L. micdadei*), but hybridizes only weakly.

Legionella GENUS 23S rRNA PROBES

Legionella Genus Probe 2926 (31mer 58% G+C) (SEQ ID NO:23)
5'-TGT CCG ACC GTA CCG AGG GTA CCT TTG TGC T-3'

Legionella Genus Probe 2930 (33mer 55% G+C) (SEQ ID NO:24)
5'-CGG TAC GGT TCT CTG TAA GTT ATG GCT AGC GGC-3'

Legionella GENUS 16S rRNA PROBES

Legionella Genus Probe 2701 (32mer, 59% G + C) (SEQ ID NO:25)
5'-TCG GAC GCA GGC TAA TCT TAA AGC GCC AGG CC- 3'

Legionella Genus Probe 2696 (34mer, 40% G+C) (SEQ ID NO:26)
5'-TTC ATA TGG CCA ACA GCT AGT TGA CAT CGT TTA C-3'

Legionella Genus Probe 2695 (30mer 57% G+C) (SEQ ID NO:27)
5'-TGT CAG TAT TAG GCC AGG TAG CCG CCT TCG-3'

The probes of the present invention may be used in a "sandwich" assay. As shown in Figure 1, the "sandwich" assay involves the use of a pair of probes simultaneously. One probe, designated the "capture" probe 12 is a bifunctional nucleotide made by adding a homopolymeric 3' tail to a probe with preferably high target specificity. The tail will hybridize to the complementary homopolymer 11 on a solid surface 10, such as a glass bead or a filter disc. Hybridization of the capture probe 12 to its target 15, in this case *Legionella* rRNA, would complex the target 15 with the solid support 10. The detector probe 13, preferably with some degree of specificity, would be a part of a detection scheme which may use virtually any sort of detection moiety 14, including

radioactivity, fluorescence, chemiluminescence, color or other detector moiety. The detector probe may be incorporated as an RNA sequence into an amplifiable Q-beta midivariant as described by Kramer and Lizardi, 1989 Nature 339, which is hereby incorporated by reference.

An environmental sample or clinical sample, such as a swab, sputum, or tissue is processed as to liberate the total nucleic acid content. The sample, putatively containing disrupted legionellae is incubated in the presence of a capture probe, detector probe, and magnetic particle beads which have been derivatized with oligo-deoxy Thymidine in a chaotropic buffer such as guanidinium isothiocyanate.

If target molecules (for example, *Legionella sp.* rRNAs) are present, a Bead-Capture Probe-Target-Detector Probe hybridization complex is formed. The presence of a magnet near the bottom of the reaction tube will cause the magnetic particle-hybridization complex to adhere to the side of the tube, enabling the removal of the sample matrix, unbound probe, and other constituents not hybridized. Repeated rehydration and denaturation of the Bead-Capture Probe-Target-Detector Probe complex would enable significant background reduction. The final detection may involve spotting the beads on a membrane and assaying by an appropriate method, such as auto-radiography, if the detector probe was labelled with a radioisotope. Alternatively, the detector probe may be an amplifiable midivariant probe.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Dot-Blot Analysis of Probe Hybridization Behavior

Dot-blot analysis, in accordance with well-known procedures, involves immobilizing a nucleic acid or a population of nucleic acids on a filter such as nitrocellulose, nylon or other derivatized membranes which can readily be obtained commercially. Either DNA or RNA can be so immobilized and subsequently tested for hybridization under a variety of conditions (stringencies) with nucleotide sequences or probes of interest. Under stringent conditions, probes with nucleotide sequences with

greater complementarity to the target will exhibit a higher level of hybridization than probes whose sequences have less homology.

Probes of the present invention are tested in a dot-blot. One hundred nanograms of RNA, is purified by phenol extraction and centrifugation through cesium trifluoroacetate gradients, denatured and spotted on a nylon membrane. Probes are isotopically labelled with the addition of a 32P-Phosphorous moiety to the 5' end of the oligonucleotide by the established polynucleotide kinase reaction. Hybridization of the probes takes place overnight at a temperature of 60°C with 10 ml 6 X SSPE, 0.3% SDS, and 10^7 CPM Probe. Unhybridized probe is removed by washing with 0.5X SSC and 0.1% SDS at 60°C for twenty minutes. The filters are then exposed to X-ray film at -70°C, and the intensity of the hybridization signals is evaluated after three hours of autoradiographic exposure.

The following is a summary of results.

TABLE 1

PROBES TARGETING 16S rRNA

Probe 2701: All *Legionella* species tested.
Probe 2696: All *Legionella* species tested, but weakly to *L. micdadei.*
Probe 2698: All *Legionella* species tested except *L. bozemanii.*
Probe 2695: All *Legionella* species tested.
Probe 2693: All *Legionella* species tested except *L. micdadei.* Hybridization to *Acholeplasma laidlawii* believed to be an error in experimental manipulation.
Probe 2699: *L. micdadei* specific
Probe 2703: *L. micdadei* and *L. dumoffii*
Probe 2704: *L. pneumophila* specific (all serotypes tested).
Probe 2705: *L. pneumophila* specific (all serotypes tested).
Probe 2708: *L. pneumophila* specific (all serotypes tested).
Probe 2690: *L. pneumophila* specific (all serotypes tested).
Probe 2697: *L. pneumophila* and three other *Legionella* species.

PROBES TARGETING 23S rRNA

Probe 2924: Sporadic hybridization within the genus.
Probe 2926: All *Legionella* species tested.
Probe 2930: All *Legionella* species tested.
Probe 2931: All *Legionella* species tested.
Probe 2932: *L. micdadei* specific.
Probe 2956: *L. micdadei* specific.
Probe 2958: *L. micdadei* specific.
Probe 2963: *L. micdadei* specific, but weak hybridizations.
Probe 2968: *L. micdadei* specific.
Probe 2928: All *Legionella* and many other eubacteria.
Probe 2957: *L. pneumophila*, very weak hybridization.
Probe 2927: All *Legionella* and many other eubacteria.
Probe 2929: A subset of *L. pneumophila* strains tested; interestingly only one of the serotype 1 strains was positive.
Probe 2954: A subset of the *L. pneumophila* strains tested.
Probe 2955: All legionellae except *L. micdadei* and *L. bozemanii*. Also hybridizes with *Neisseriae*.

The data from the dot blot assay are presented below as TABLE 2. In this table, ++++ indicates the strongest signals observed; +++ indicates a strong signal was observed; ++ indicates a somewhat weaker, but definitely positive hybridization signal observed; + indicates a weak signal; +- indicates a very weak, virtually absent, barely detectable signal; - indicates no signal observed. If a probe binds strongly (either ++++ or +++) to at least one target, but only exhibits a weak hybridization (+ or +-) to a second target, the probe is considered to substantially hybridize only with the targets giving the ++++ or +++ results.

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION

Genus species	strain	2701	2696	2698	2695	2693
Legionella pneumophila	33152	++++	+	++++	++++	++++
Legionella pneumophila	33153	++++	+	++++	++++	++++
Legionella pneumophila	33154	++++	+	++++	++++	++++
Legionella pneumophila	33155	++++	+	++++	++++	++++
Legionella pneumophila	33156	++++	+	++++	++++	++++
Legionella pneumophila	33216	++++	+	++++	++++	++++
Legionella pneumophila	33215	++++	+	++++	++++	++++
Legionella pneumophila	33823	++++	+	++++	++++	++++
Legionella pneumophila	35096	++++	+	++++	++++	++++
Legionella bozemanii	33217	++++	+	+	++++	++++
Legionella dumoffii	33279	++++	+	++++	++++	++++
Legionella gormanii	33297	++++	+	++++	++++	++++
Legionella longbeachae	33462	++++	+	++++	++++	++++
Legionella langbeachae	33484	++++	+	++++	++++	++++
Legionella micdadei	33204	++++	-	++++	++++	-
Acholeplasma laidlawii		-	-	-	-	++++
Acinetobacter calcoaceticus	GT0002	-	-	-	-	-
Actinobacillus actinomycetemcomitans	29522	-	-	-	-	+
Aeromonas sobria	GT0007	-	-	-	-	-
Alteromonas putrefaciens	GT1945	-	-	-	-	+
Citrobacter diversus	GT0030	-	-	-	-	-
Citrobacter freundii	GT0687	-	-	-	-	-
Edwardsiella tarda	GT0569	-	-	-	-	-
Enterobacter agglomerans	GT3130	-	-	+-	-	-
Escherichia coli	GT1592	-	-	-	-	-
Escherichia coli	GT1659	-	-	-	-	-
Haemophilus influenza	ATCC 33391	-	-	-	-	-
Haemophilus parainfluenza	NCTC 7901	-	-	-	-	-
Haemophilus pleuropneumoniae	27088	-	-	-	-	-
Hafnia alvei	GT0241	-	-	-	-	-
Klebsiella pneumoniae	GT1500	-	-	-	-	-
Morganella morganii	25830	-	-	-	-	-
Pasteurella aerogenes	27883	-	-	-	-	-
Pasteurella pneumotropica	NCTC 8141	-	-	-	-	-
Plesiomonas shigelloides	14029	-	-	-	-	-
Proteus mirabilis	GT1496	-	-	-	-	-
Providencia alcalifaciens	GT0371	-	-	-	-	-
Salmonella typhimurium	GT0389	-	-	-	-	-
Serratia marcescens	GT0392	-	-	-	-	-
Shigella flexneri	12022	-	-	-	-	-
Pseudomonas aeruginosa	27853	-	-	-	-	++++
Vibrio parahaemolyticus	GT0568	-	-	-	-	-
Xanthomonas maltophilia	GT0417	-	-	-	-	-
Yersinia enterocolitica	GT0419	-	-	-	-	-
Yersinia pseudotuberculosis	29833	-	-	-	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2701	2696	2698	2695	2693
Neisseria gonorrhoeae	GT0315	-	-	-	-	-
Neisseria meningitidis	GT0349	-	-	-	-	-
Agrobacterium tumefaciens	GT2021	-	-	-	-	-
Desulfovibrio desulfuricans	ATCC 7757	-	-	-	-	-
Francisella tularensis	GT2172	-	-	-	-	-
Campylobacter jejuni	33560	-	-	-	-	-
Bacillus subtilis	GT0804	-	-	-	-	-
Clostridium perfringens	ATCC 13124	-	-	-	-	-
Mycoplasma pneumoniae	PI1428	-	-	-	-	-
Mycoplasma hominis	PG-21	-	-	-	-	-
Ureaplasma urealyticum	#8	-	-	-	-	-
Mycoplasma genitalium	G-37	-	-	-	-	-
Staphlyococcus aureus	GT2047	-	-	-	-	-
Streptococcus pneumoniae	GT0408	-	-	-	-	-
Streptococcus salivarius	GT0410	-	-	-	-	-
Bifidobacterium dentium	GT0012	-	-	-	-	-
Corynebacterium genitalium	G45	-	-	-	-	-
Corynebacterium glutamicum	GT2120	-	-	-	-	-
Corynebacterium pseudotuberculosis	GT2122	-	-	-	-	-
Mycobacterium kansasii		-	-	-	-	-
Mycobacterium tuberculosis	GT2487	-	-	-	-	-
Mycobacterium avium	GT3246	-	-	-	-	-
Spirochaeta aurantia		-	-	-	-	-
Bacteroides fragilis	25285	-	-	-	-	-
Bacteroides fragilis	29771	-	-	-	-	-
Chlamydia pneumoniae	(TWAR)	-	-	-	-	-
Normal Stool RNA		-	-	-	-	-
Wheat Germ		-	-	-	-	-
Normal Human DNA	Caski	-	-	-	-	-
Candida albicans	11006	-	-	-	-	-
Aspergillus flavus	10124	-	-	-	-	-
Blastomyces dermatidis	60193	-	-	-	-	-
Cryptococcus neoformans	14116	-	-	-	-	-
Saccharomyces cerevisiae	18824	-	-	-	-	-
Coxiella burnetii	PCR	-	-	+-	-	+-
Legionella pneumophila	PCR	++++	+	++++	++++	++++
Wolbachia persica	PCR	-	-	-	-	+-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2699	2703
Legionella pneumophila	33152	-	-
Legionella pneumophila	33153	-	-
Legionella pneumophila	33154	-	-
Legionella pneumophila	33155	-	-
Legionella pneumophila	33156	-	-
Legionella pneumophila	33216	-	-
Legionella pneumophila	33215	-	-
Legionella pneumophila	33823	-	-
Legionella pneumophila	35096	-	-
Legionella bozemanii	33217	-	-
Legionella dumoffii	33279	-	++++
Legionella gormanii	33297	-	-
Legionella longbeachae	33462	-	-
Legionella langbeachae	33484	-	-
Legionella micdadei	33204	++++	++++
Acholeplasma laidlawii		-	-
Acinetobacter calcoaceticus	GT0002	-	-
Actinobacillus actinomycetemcomitans	29522	-	-
Aeromonas sobria	GT0007	-	-
Alteromonas putrefaciens	GT1945	-	-
Citrobacter diversus	GT0030	-	-
Citrobacter freundii	GT0687	-	-
Edwardsiella tarda	GT0569	-	-
Enterobacter agglomerans	GT3130	-	-
Escherichia coli	GT1592	-	-
Escherichia coli	GT1659	-	-
Haemophilus influenza	ATCC 33391	-	-
Haemophilus parainfluenza	NCTC 7901	-	-
Haemophilus pleuropneumoniae	27088	-	-
Hafnia alvei	GT0241	-	-
Klebsiella pneumoniae	GT1500	-	-
Morganella morganii	25830	-	-
Pasteurella aerogenes	27883	-	-
Pasteurella pneumotropica	NCTC 8141	-	-
Plesiomonas shigelloides	14029	-	-
Proteus mirabilis	GT1496	-	-
Providencia alcalifaciens	GT0371	-	-
Salmonella typhimurium	GT0389	-	-
Serratia marcescens	GT0392	-	-
Shigella flexneri	12022	-	-
Pseudomonas aeruginosa	27853	-	-
Vibrio parahaemolyticus	GT0568	-	-
Xanthomonas maltophilia	GT0417	-	-
Yersinia enterocolitica	GT0419	-	-
Yersinia pseudotuberculosis	29833	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2699	2703
Neisseria gonorrhoeae	GT0315	-	-
Neisseria meningitidis	GT0349	-	-
Agrobacterium tumefaciens	GT2021	-	-
Desulfovibrio desulfuricans	ATCC 7757	-	-
Francisella tularensis	GT2172	-	-
Campylobacter jejuni	33560	-	-
Bacillus subtilis	GT0804	-	-
Clostridium perfringens	ATCC 13124	-	-
Mycoplasma pneumoniae	PI1428	-	-
Mycoplasma hominis	PG-21	-	-
Ureaplasma urealyticum	#8	-	-
Mycoplasma genitalium	G-37	-	-
Staphlyococcus aureus	GT2047	-	-
Streptococcus pneumoniae	GT0408	-	-
Streptococcus salivarius	GT0410	-	-
Bifidobacterium dentium	GT0012	-	-
Corynebacterium genitalium	G45	-	-
Corynebacterium glutamicum	GT2120	-	-
Corynebacterium pseudotuberculosis	GT2122	-	-
Mycobacterium kansasii		-	-
Mycobacterium tuberculosis	GT2487	-	-
Mycobacterium avium	GT3246	-	-
Spirochaeta aurantia		-	-
Bacteroides fragilis	25285	-	-
Bacteroides fragilis	29771	-	-
Chlamydia pneumoniae	(TWAR)	-	-
Normal Stool RNA		-	-
Wheat Germ		-	-
Normal Human DNA	Caski	-	-
Candida albicans	11006	-	-
Aspergillus flavus	10124	-	-
Blastomyces dermatidis	60193	-	-
Cryptococcus neoformans	14116	-	-
Saccharomyces cerevisiae	18824	-	-
Coxiella burnetii	PCR	-	-
Legionella pneumophila	PCR	-	-
Wolbachia persica	PCR	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2704	2705	2708	2690	2697
Legionella pneumophila	33152	++	++++	++++	++++	++++
Legionella pneumophila	33153	++	++++	++++	++++	++++
Legionella pneumophila	33154	+++	++++	++++	++++	++++
Legionella pneumophila	33155	++++	++++	++++	++++	++++
Legionella pneumophila	33156	++++	++++	++++	++++	++++
Legionella pneumophila	33216	++++	++++	++++	++++	++++
Legionella pneumophila	33215	++++	++++	++++	+++	++++
Legionella pneumophila	33823	++++	++++	++++	+++	++++
Legionella pneumophila	35096	++++	++++	++++	+++	++++
Legionella bozemanii	33217	-	-	-	-	++++
Legionella dumoffii	33279	-	-	-	-	++++
Legionella gormanii	33297	-	-	-	-	++++
Legionella longbeachae	33462	-	-	-	-	-
Legionella langbeachae	33484	-	-	-	-	-
Legionella micdadei	33204	-	-	-	-	-
Acholeplasma laidlawii		-	-	-	-	-
Acinetobacter calcoaceticus	GT0002	-	-	-	-	-
Actinobacillus actinomycetemcomitans	29522	-	-	-	-	-
Aeromonas sobria	GT0007	-	-	-	-	-
Alteromonas putrefaciens	GT1945	-	-	-	-	-
Citrobacter diversus	GT0030	-	-	-	-	-
Citrobacter freundii	GT0687	-	-	-	-	-
Edwardsiella tarda	GT0569	-	-	-	-	-
Enterobacter agglomerans	GT3130	-	-	-	-	-
Escherichia coli	GT1592	-	-	-	-	-
Escherichia coli	GT1659	-	-	-	-	-
Haemophilus influenza	ATCC 33391	-	-	-	-	-
Haemophilus parainfluenza	NCTC 7901	-	-	-	-	-
Haemophilus pleuropneumoniae	27088	-	-	-	-	-
Hafnia alvei	GT0241	-	-	-	-	-
Klebsiella pneumoniae	GT1500	-	-	-	-	-
Morganella morganii	25830	-	-	-	-	-
Pasteurella aerogenes	27883	-	-	-	-	-
Pasteurella pneumotropica	NCTC 8141	-	-	-	-	-
Plesiomonas shigelloides	14029	-	-	-	-	-
Proteus mirabilis	GT1496	-	-	-	-	-
Providencia alcalifaciens	GT0371	-	-	-	-	-
Salmonella typhimurium	GT0389	-	-	-	-	-
Serratia marcescens	GT0392	-	-	-	-	-
Shigella flexneri	12022	-	-	-	-	-
Pseudomonas aeruginosa	27853	-	-	-	-	-
Vibrio parahaemolyticus	GT0568	-	-	-	-	-
Xanthomonas maltophilia	GT0417	-	-	-	-	-
Yersinia enterocolitica	GT0419	-	-	-	-	-
Yersinia pseudotuberculosis	29833	-	-	-	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2704	2705	2708	2690	2697
Neisseria gonorrhoeae	GT0315	-	-	-	-	-
Neisseria meningitidis	GT0349	-	-	-	-	-
Agrobacterium tumefaciens	GT2021	-	-	-	-	-
Desulfovibrio desulfuricans	ATCC 7757	-	-	-	-	-
Francisella tularensis	GT2172	-	-	-	-	-
Campylobacter jejuni	33560	-	-	-	-	-
Bacillus subtilis	GT0804	-	-	-	-	-
Clostridium perfringens	ATCC 13124	-	-	-	-	-
Mycoplasma pneumoniae	PI1428	-	-	-	-	-
Mycoplasma hominis	PG-21	-	-	-	-	-
Ureaplasma urealyticum	#8	-	-	-	-	-
Mycoplasma genitalium	G-37	-	-	-	-	-
Staphlyococcus aureus	GT2047	-	-	-	-	-
Streptococcus pneumoniae	GT0408	-	-	-	-	-
Streptococcus salivarius	GT0410	-	-	-	-	-
Bifidobacterium dentium	GT0012	-	-	-	-	-
Corynebacterium genitalium	G45	-	-	-	+-	-
Corynebacterium glutamicum	GT2120	-	-	-	-	-
Corynebacterium pseudotuberculosis	GT2122	-	-	-	-	-
Mycobacterium kansasii		-	-	-	-	-
Mycobacterium tuberculosis	GT2487	-	-	-	+-	-
Mycobacterium avium	GT3246	-	-	-	-	-
Spirochaeta aurantia		-	-	-	-	-
Bacteroides fragilis	25285	-	-	-	-	-
Bacteroides fragilis	28771	-	-	-	-	-
Chlamydia pneumoniae	(TWAR)		-	-	-	-
Normal Stool RNA		-	-	-	-	-
Wheat Germ		-	-	-	-	-
Normal Human DNA	Caski	-	-	-	-	-
Candida albicans	11006	-	-	-	-	-
Aspergillus flavus	10124	-	-	-	-	-
Blastomyces dermatidis	60193	-	-	-	-	-
Cryptococcus neoformans	14116	-	-	-	-	-
Saccharomyces cerevisiae	18824	-	-	-	-	-
Coxiella burnetii	PCR	-	+-	-	+-	-
Legionella pneumophila	PCR	++++	++++	++++	++++	++++
Wolbachia persica	PCR	-	-	-	+-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2924	2926	2930
Legionella pneumophila	33152	++	++++	++++
Legionella pneumophila	33153	+++	++++	++++
Legionella pneumophila	33154	+++	++++	++++
Legionella pneumophila	33155	++	++++	+++
Legionella pneumophila	33156	+++	++++	++++
Legionella pneumophila	33216	+++	++++	+++
Legionella pneumophila	33215	+++	++++	++++
Legionella pneumophila	33823	+++	++++	++++
Legionella pneumophila	35096	+	++++	++++
Legionella bozemanii	33217	+	++++	++++
Legionella dumoffii	33279	-	++++	++++
Legionella gormanii	33297	+	++++	++++
Legionella longbeachae	33462	++	++++	++++
Legionella langbeachae	33484	++	++++	++++
Legionella micdadei	33204	+++	++++	++++
Legionella jordanis		+	++++	++
Acholeplasma laidlawii		-	-	-
Acinetobacter calcoaceticus	GT0002	-	-	-
Actinobacillus actinomycetemcomitans	29522	-	-	-
Aeromonas sobria	GT0007	-	-	-
Alteromonas putrefaciens	GT1945	-	-	-
Citrobacter diversus	GT0030	-	-	-
Citrobacter freundii	GT0687	-	-	-
Edwardsiella tarda	GT0569	-	-	-
Enterobacter agglomerans	GT3130	-	-	-
Escherichia coli	GT1592	-	-	-
Escherichia coli	GT1659	-	-	-
Haemophilus influenza	ATCC 33391	-	-	-
Haemophilus parainfluenza	NCTC 7901	-	-	-
Haemophilus pleuropneumoniae	27088	-	-	-
Hafnia alvei	GT0241	-	-	-
Klebsiella pneumoniae	GT1500	-	-	-
Morganella morganii	25830	-	-	-
Pasteurella aerogenes	27883	-	-	-
Pasteurella pneumotropica	NCTC 8141	-	-	-
Plesiomonas shigelloides	14029	-	-	-
Proteus mirabilis	GT1496	-	-	-
Providencia alcalifaciens	GT0371	-	-	-
Salmonella typhimurium	GT0389	-	-	-
Serratia marcescens	GT0392	-	-	-
Shigella flexneri	12022	-	-	-
Pseudomonas aeruginosa	27853	-	-	-
Vibrio parahaemolyticus	GT0568	-	-	-
Xanthomonas maltophilia	GT0417	-	-	-
Yersinia enterocolitica	GT0419	-	-	-
Yersinia pseudotuberculosis	29833	-	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2924	2928	2930
Neisseria gonorrhoeae	GT0315	-	-	-
Neisseria meningitidis	GT0349	-	-	-
Agrobacterium tumefaciens	GT2021	-	-	-
Desulfovibrio desulfuricans	ATCC 7757	-	-	-
Francisella tularensis	GT2172	-	+	-
Campylobacter jejuni	33560	-	-	-
Bacillus subtilis	GT0804	-	-	-
Clostridium perfringens	ATCC 13124	-	-	-
Mycoplasma pneumoniae	PI1428	-	-	-
Mycoplasma hominis	PG-21	-	-	-
Ureaplasma urealyticum	#8	-	-	-
Mycoplasma genitalium	G-37	-	-	-
Staphlyococcus aureus	GT2047	-	-	-
Streptococcus pneumoniae	GT0408	-	-	-
Streptococcus salivarius	GT0410	-	-	-
Bifidobacterium dentium	GT0012	-	-	-
Corynebacterium genitalium	G45	-	-	-
Corynebacterium glutamicum	GT2120	-	-	-
Corynebacterium pseudotuberculosis	GT2122	-	-	-
Mycobacterium kansasii		-	-	-
Mycobacterium tuberculosis	GT2487	-	-	-
Mycobacterium avium	GT3246	-	-	-
Spirochaeta aurantia		-	-	-
Bacteroides fragilis	25285	-	-	-
Bacteroides fragilis	29771	-	-	-
Chlamydia pneumoniae	(TWAR)	-	-	-
Normal Stool RNA		-	-	-
Wheat Germ		-	-	-
Normal Human DNA	Caski	-	-	-
Candida albicans	11006	-	-	-
Aspergillus flavus	10124	-	-	-
Blastomyces dermatidis	60193	-	-	-
Cryptococcus neoformans	14116	-	-	-
Saccharomyces cerevisiae	18824	-	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2932	2956	2958	2963	2968
Legionella pneumophila	33152	-	-	-	-	-
Legionella pneumophila	33153	-	-	-	-	-
Legionella pneumophila	33154	-	-	-	-	-
Legionella pneumophila	33155	-	-	-	-	-
Legionella pneumophila	33156	-	-	-	-	-
Legionella pneumophila	33216	-	-	-	-	-
Legionella pneumophila	33215	-	-	-	-	-
Legionella pneumophila	33823	-	-	-	-	-
Legionella pneumophila	35096	-	-	-	-	-
Legionella bozemanii	33217	-	-	-	-	-
Legionella dumoffii	33279	-	-	-	-	-
Legionella gormanii	33297	-	-	-	-	-
Legionella longbeachae	33462	-	-	-	-	-
Legionella langbeachae	33484	-	-	-	-	-
Legionella micdadei	33204	++++	++++	++++	++	++++
Legionella jordanis		-	-	-	-	-
Acholeplasma laidlawii		-	-	-	-	-
Acinetobacter calcoaceticus	GT0002	-	-	-	-	-
Actinobacillus actinomycetemcomitans	29522	-	-	-	-	-
Aeromonas sobria	GT0007	-	-	-	-	-
Alteromonas putrefaciens	GT1945	-	-	-	-	-
Citrobacter diversus	GT0030	-	-	-	-	-
Citrobacter freundii	GT0687	-	-	-	-	-
Edwardsiella tarda	GT0569	-	-	-	-	-
Enterobacter agglomerans	GT3130	-	-	-	-	-
Escherichia coli	GT1592	-	-	-	-	-
Escherichia coli	GT1659	-	-	-	-	-
Haemophilus influenza	ATCC 33391	-	+	-	-	-
Haemophilus parainfluenza	NCTC 7901	-	-	-	-	-
Haemophilus pleuropneumoniae	27088	-	-	-	-	-
Hafnia alvei	GT0241	-	-	-	-	-
Klebsiella pneumoniae	GT1500	-	-	-	-	-
Morganella morganii	25830	-	-	-	-	-
Pasteurella aerogenes	27883	-	-	-	-	-
Pasteurella pneumotropica	NCTC 8141	-	-	-	-	-
Plesiomonas shigelloides	14029	-	-	-	-	-
Proteus mirabilis	GT1496	-	-	-	-	-
Providencia alcalifaciens	GT0371	-	-	-	-	-
Salmonella typhimurium	GT0389	-	-	-	-	-
Serratia marcescens	GT0392	-	-	-	-	-
Shigella flexneri	12022	-	-	-	-	-
Pseudomonas aeruginosa	27853	-	-	-	-	-
Vibrio parahaemolyticus	GT0568	-	-	-	-	-
Xanthomonas maltophilia	GT0417	-	-	-	-	-
Yersinia enterocolitica	GT0419	-	-	-	-	-
Yersinia pseudotuberculosis	29833	-	-	-	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2932	2956	2958	2963	2968
Neisseria gonorrhoeae	GT0315	-	-	-	-	-
Neisseria meningitidis	GT0349	-	-	-	-	-
Agrobacterium tumefaciens	GT2021	-	-	-	-	-
Desulfovibrio desulfuricans	ATCC 7757	-	-	-	-	-
Francisella tularensis	GT2172	-	-	-	-	-
Campylobacter jejuni	33560	-	-	-	-	-
Bacillus subtilis	GT0804	-	-	-	-	-
Clostridium perfringens	ATCC 13124	-	-	-	-	-
Mycoplasma pneumoniae	PI1428	-	-	-	-	-
Mycoplasma hominis	PG-21	-	-	-	-	-
Ureaplasma urealyticum	#8	-	-	-	-	-
Mycoplasma genitalium	G-37	-	-	-	-	-
Staphlyococcus aureus	GT2047	-	-	-	-	-
Streptococcus pneumoniae	GT0408	-	-	-	-	-
Streptococcus salivarius	GT0410	-	-	-	-	-
Bifidobacterium dentium	GT0012	-	-	-	-	-
Corynebacterium genitalium	G45	-	-	-	-	-
Corynebacterium glutamicum	GT2120	-	-	-	-	-
Corynebacterium pseudotuberculosis	GT2122	-	-	-	-	-
Mycobacterium kansasii		-	-	-	-	-
Mycobacterium tuberculosis	GT2487	-	-	-	-	-
Mycobacterium avium	GT3246	-	-	-	-	-
Spirochaeta aurantia		-	-	-	-	-
Bacteroides fragilis	25285	-	-	-	-	-
Bacteroides fragilis	29771	-	-	-	-	-
Chlamydia pneumoniae	(TWAR)	-	-	-	-	-
Normal Stool RNA		-	-	-	-	-
Wheat Germ		-	-	-	-	-
Normal Human DNA	Caski	-	-	-	-	-
Candida albicans	11006	-	-	-	-	-
Aspergillus flavus	10124	-	-	-	-	-
Blastomyces dermatidis	60193	-	-	-	-	-
Cryptococcus neoformans	14116	-	-	-	-	-
Saccharomyces cerevisiae	18824	-	-	-	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2928	2957	2927	2929	2954	2955	2959
Legionella pneumophila	33152	++++	+-	++++	++++	++++	++++	++++
Legionella pneumophila	33153	++++	+-	++++	+-	++++	++++	++++
Legionella pneumophila	33154	++++	+-	++++	++++	++++	++++	++++
Legionella pneumophila	33155	++++	+-	++++	+-	++++	++++	-
Legionella pneumophila	33156	++++	+-	++++	-	+	++++	++++
Legionella pneumophila	33216	++++	+-	++++	-	+	++++	++++
Legionella pneumophila	33215	++++	+-	++++	++++	++++	++++	++++
Legionella pneumophila	33823	++++	+-	++++	+-	++++	++++	-
Legionella pneumophila	35096	++++	+-	++++	++++	++++	++++	++++
Legionella bozemanii	33217	++++	-	++++	-	-	+-	-
Legionella dumoffii	33279	+++	-	++++	-	-	++++	++++
Legionella gormanii	33297	++++	-	++++	-	-	++++	+-
Legionella longbeachae	33462	++++	-	++++	-	-	++++	-
Legionella langbeachae	33484	++++	-	++++	-	-	++++	-
Legionella micdadei	33204	++++	-	++++	-	-	+-	-
Legionella jordanis		+++	-	++++	-	-	++++	-
Acholeplasma laidlawii		-	-	-	-	-	-	-
Acinetobacter calcoaceticus	GT0002	-	-	++++	-	-	-	-
Actinobacillus actinomycetemcomitans	29522	-	-	-	-	-	-	-
Aeromonas sobria	GT0007	-	-	+++	-	-	-	-
Alteromonas putrefaciens	GT1945	-	-	-	-	-	-	-
Citrobacter diversus	GT0030	-	-	-	-	-	-	-
Citrobacter freundii	GT0687	-	-	-	-	-	-	-
Edwardsiella tarda	GT0569	+	-	+++	-	-	-	-
Enterobacter agglomerans	GT3130	+-	-	-	-	-	-	-
Escherichia coli	GT1592	-	-	-	-	-	-	-
Escherichia coli	GT1659	-	-	-	-	-	-	-
Haemophilus influenza	ATCC 33391	-	-	-	-	-	-	-
Haemophilus parainfluenza	NCTC 7901	-	-	-	-	-	-	-
Haemophilus pleuropneumoniae	27088	-	-	-	-	-	-	-
Hafnia alvei	GT0241	++++	-	++	-	-	-	-
Klebsiella pneumoniae	GT1500	-	-	-	-	-	-	-
Morganella morganii	25830	++++	-	-	-	-	-	-
Pasteurella aerogenes	27883	-	-	-	-	-	-	-
Pasteurella pneumotropica	NCTC 8141	-	-	-	-	-	-	-
Plesiomonas shigelloides	14029	-	-	+	-	-	-	-
Proteus mirabilis	GT1496	++++	-	++	-	-	-	-
Providencia alcalifaciens	GT0371	++++	-	++	-	-	-	-
Salmonella typhimurium	GT0389	-	-	+	-	-	-	-
Serratia marcescens	GT0392	++++	-	++	-	-	-	-
Shigella flexneri	12022	-	-	-	-	-	-	-
Pseudomonas aeruginosa	27853	-	-	-	-	-	-	-
Vibrio parahaemolyticus	GT0568	-	-	+++	-	-	-	-
Xanthomonas maltophilia	GT0417	-	-	-	-	-	-	-
Yersinia enterocolitica	GT0419	++++	-	+++	-	-	-	-
Yersinia pseudotuberculosis	29833	++++	-	+++	-	-	-	-

TABLE 2. EXPERIMENTALLY DETERMINED PROBE HYBRIDIZATION (continued)

Genus species	strain	2928	2957	2927	2929	2954	2955	2959
Neisseria gonorrhoeae	GT0315	-	-	-	-	-	++++	-
Neisseria meningitidis	GT0349	-	-	-	-	-	+-	-
Agrobacterium tumefaciens	GT2021	-	-	-	-	-	-	-
Desulfovibrio desulfuricans	ATCC 7757	-	-	-	-	-	-	-
Francisella tularensis	GT2172	++++	-	-	-	-	-	-
Campylobacter jejuni	33560	-	-	-	-	-	-	-
Bacillus subtilis	GT0804	-	-	-	-	-	-	-
Clostridium perfringens	ATCC 13124	-	-	-	-	-	-	-
Mycoplasma pneumoniae	PI1428	-	-	-	-	-	-	-
Mycoplasma hominis	PG-21	-	-	++	-	-	-	-
Ureaplasma urealyticum	#8	-	-	-	-	-	-	-
Mycoplasma genitalium	G-37	-	-	-	-	-	-	-
Staphlyococcus aureus	GT2047	-	-	-	-	-	-	-
Streptococcus pneumoniae	GT0408	-	-	-	-	-	-	-
Streptococcus salivarius	GT0410	-	-	-	-	-	-	-
Bifidobacterium dentium	GT0012	-	-	-	-	-	-	-
Corynebacterium genitalium	G45	-	-	-	-	-	-	-
Corynebacterium glutamicum	GT2120	-	-	+-	-	-	-	-
Corynebacterium pseudotuberculosis	GT2122	-	-	-	-	-	-	-
Mycobacterium kansasii		-	-	-	-	-	-	-
Mycobacterium tuberculosis	GT2487	-	-	-	-	-	-	-
Mycobacterium avium	GT3246	-	-	-	-	-	-	-
Spirochaeta aurantia		-	-	-	-	-	-	-
Bacteroides fragilis	25285	-	-	-	-	-	-	-
Bacteroides fragilis	29771	-	-	-	-	-	-	-
Chlamydia pneumoniae	(TWAR)	-	-	-	-	-	-	-
Normal Stool RNA		-	-	-	-	-	-	-
Wheat Germ		-	-	-	-	-	-	-
Normal Human DNA	Caski	-	-	-	-	-	-	-
Candida albicans	11006	-	-	-	-	-	-	-
Aspergillus flavus	10124	-	-	-	-	-	-	-
Blastomyces dermatidis	60193	-	-	-	-	-	-	-
Cryptococcus neoformans	14116	-	-	-	-	-	-	-
Saccharomyces cerevisiae	18824	-	-	-	-	-	-	-

HYBRIDIZATION = 10ML of 6X SSPE, 0.3% SDS, 10E7 CPM PROBE. 60°C OVERNIGHT
WASH = 0.5X SSC, 0.1% SDS AT 60°C FOR 20MIN.
EXPOSURE = X-RAY FILM -70°C 3HRS.

Example 2

Dual Probe Hybridization

The following probe pairs are used in a sandwich assay:

Legionella genus 16S rRNA: Probe 2701 + Probe 1660 (Probe 1660 will bind with all bacterial species tested to date. It is described in PCT application WO90/15157, which is hereby incorporated b y reference).

Probe 2695 + Probe 2696

L. pneumophila 16S rRNA: Probe 2704 + Probe 2697
Probe 2705 + Probe 2690

L. micdadei 23S rRNA: Probe 2958 + 2968
Probe 2956 + 2958

Legionella genus 23S rRNA: Probe 2926 + Probe 2931

L. micdadei 16S rRNA: Probe 2699 + Probe 2695

In each instance, target organism was detected, and non-target DNA was not detected.

Example 3

Clinical Diagnosis of Legionellosis

A sample from an individual suspected of having legionellosis is processed to yield DNA. A probe of this invention is used in conjunction with the antiparallel complement of a second probe of this invention to enzymatically amplify a segment of *L. pneumophila* or *L. micadei* gene encoding legionella rRNA in a polymerase chain reaction. Resultant material is then assayed in a sandwich assay. The polymerase chain reaction can, itself be made either highly specific by employing probe/primers described herein, or the reaction may be made more general using probes such as those described in copending

USSN 359,158 which is hereby incorporated by reference, and then identifying the amplification product as *L. pneumophila* using a sandwich assay.

Example 4

In situ Hybridization as a Cytological Stain

The probes of this invention may be used as cytological staining reagents. A sample, such as a sputum sample is applied to a microscope slide. After fixation and lysis, hybridization of probes is carried out in situ. For example, Probe 2705 (hybridizes to all serotypes of *L. pneumophila*) is labelled with a florescent label and used to stain the specimen. If *L. pneumophila* is present in the sample, small fluorescent bodies will be visible under a fluorescent microscope.

Example 5

Confirmation of Presence of *Legionella* sp. Following Culture

Following a standard cultivation step for *Legionella*, such as on buffered charcoal yeast extract agar plate or in liquid culture enrichment, the presence of *Legionella* is tested for. One method is by use of the sandwich assay described in Example 2. Pure culture is not necessary.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: AMOCO CORPORATION

(ii) TITLE OF INVENTION: Nucleic Acid Probes for the Detection of Bacteria of the Genus Legionella and Methods for the Detection of the Etiological Agents of Legionnaires' Disease

(iii) NUMBER OF SEQUENCES: 27

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 55 Shuman Blvd., Suite 600
(C) CITY: Naperville
(D) STATE: IL
(E) COUNTRY: USA
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Norval B. Galloway
(B) REGISTRATION NUMBER: 33,595
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(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (708) 717-2447
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCGCCATCTG TCTAGCAAGC TAGACAATGC T 31

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACTTTTAAGG ATTTGCTCCA GGTCGCCCCT 30

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACTACGACCG ACTTTTAAGG ATTTGCTCCA G 31

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TAGAGTCCCC ACCATCACAT GCTGGCAACT AAGGAT 36

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCAATGACTT CTCTATACCA AAAGGGTCAG AACCAC 36

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTCTATCGCC AACTTTCCCA AATTGTTCTA C 31

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCACCTCAGA GTTATGGAAA ACCGGATTTG C 31

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTCGTCACTA ACCTCATTCA TAAGGCCAAC AACT 34

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGTATCGTG GTACTTCCCA GAACCTTCTA C 31

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCCACCTCT CAGTGAACCT TCTTCAGCCT 30

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCACCTCAGC CTTAACAAGG GGCCGGATTT GC 32

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCTCTTCAGC TCATTAAGCA TGTCAATTCA CC 32

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCAATGACTT CTCCGCACAC CGTAGTGTCA GACCAC 37

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCGCCACCCA TCTAGTAAAC TAGACCGTGC T 31

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTTCCCCAAG TTGTCCCCCT CTTCAAGGCA TAT 33

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TCTTAACCTA TCAACCCTCC TCCCCACTAG AAG 33

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGGCGGTCAA CTTATCGCGT TTGCTGCGCC 30

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TAAGACCAAC TTTCGTTCCT GCTCGAGCCG T 31

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCAGACTCGA TTTCTCTACG GCTCCCTTAT 30

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCACACTTCT CAATGCACCT TCATCAGCCT 30

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CACAGTCATC ATCAAAGTCC AGTGCAAAAC T 31

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCTCTCCAGC TCTGAAAGTA AATCCCATCA CC 32

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TGTCCGACCG TACCGAGGGT ACCTTTGTGC T 31

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGGTACGGTT CTCTGTAAGT TATGGCTAGC GGC 33

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCGGACGCAG GCTAATCTTA AAGCGCCAGG CC 32

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTCATATGGC CAACAGCTAG TTGACATCGT TTAC 34

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TGTCAGTATT AGGCCAGGTA GCCGCCTTCG 30

What is claimed is:

1. A probe comprising: a nucleic acid consisting essentially of 10 to 250 nucleotides which hybridizes preferentially to 23S or 16S rRNA or rDNA of at least one target *Legionella* bacteria, the target *Legionella* bacteria selected from the group consisting of:
   a) *L. pneumophila* bacteria;
   b) *L. micdadei* bacteria;
   c) a *Legionella* cluster;
   d) a *L. pneumophila* cluster

   and optionally, a detection or enhancement moiety.

2. A probe according to claim 1 which hybridizes preferentially to 23S rRNA.

3. A probe according to claim 1 which hybridizes preferentially to 16S rRNA.

4. A probe which is complementary to or at least 90% homologous with a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 2701, 2703, 2704, 2705, 2697, 2690, 2698, 2695, 2693, 2708, 2699, 2924, 2926, 2930, 2932, 2956, 2958, 2963, 2968, 2928, 2957, 2927, 2929, 2954, 2955, and 2959.

5. A nucleic acid consisting essentially of 10 to 250 nucleotides which hybridizes preferentially to 23S or 16S rRNA or rDNA of at least one target *Legionella* bacteria, the target *Legionella* bacteria selected from the group consisting of:
   a) *L. pneumophila* bacteria;
   b) *L. micdadei* bacteria; and
   c) a *Legionella* cluster;
   d) a *L. pneumophila* cluster.

6. A nucleic acid which is complementary to or at least 90% homologous with a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 2701, 2703, 2704, 2705, 2697, 2690, 2698, 2695, 2696, 2693, 2708, 2699, 2924, 2926, 2930, 2932, 2956, 2958, 2963, 2968, 2928, 2957, 2927, 2929, 2954, 2955, and 2959.

7. A method of detecting the presence of *L. pneumophila* in a sample suspected of containing *L. pneumophila* comprising:

a) contacting the sample with at least one nucleic acid composition having 10 to 250 nucleotides which, under conditions that allow said nucleic acid to hybridize, hybridize preferentially with rRNA or rDNA of *L. pneumophila*;

b) imposing hybridization conditions on the sample to form a hybridization product in the presence of *L. pneumophila*; and

c) detecting the hybridization product as an indication of the presence of *L. pheumophila*.

8. A method according to claim 7 wherein the nucleic acid composition which is complementary to or at least 90% homologous with a sequence comprising any ten consecutive nucleotides selected from the group consisting of sequences defined by probes 2704, 2705, 2708, 2690, 2957, 2929, and 2954.

9. A method according to claim 7 wherein the contacting step comprises a first nucleic acid and a second nucleic acid, each nucleic acid having a different sequence, and complementary to or at least 90% homologous with any ten consecutive nucleotides selected from the group consisting of sequences defined by probes 2704, 2705, 2708, 2690, 2957, 2929, and 2954.

10. A method according to claim 9 wherein the first nucleic acid and second nucleic acid are selected from the group consisting of probe sets: 2704 and 2697, and 2705 and 2690.

11. A method of detecting the presence of *L. micdadei* in a sample suspected of containing *L. micdadei* comprising:

a) contacting the sample with at least one nucleic acid composition having 10 to 250 nucleotides which, under conditions that allow said nucleic acid to hybridize, hybridize preferentially with rRNA and rDNA of *L. micdadei*;

b) imposing hybridization conditions on the sample to form a hybridization product in the presence of *L. micdadei*; and

c) detecting the hybridization product as an indication of the presence of *L. micdadei*.

12. A method according to claim 11 wherein the nucleic acid composition which is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides selected from the group consisting of sequences defined by probes 2699, 2932, 2956, 2958, 2963, 2968, and 2703.

13. A method according to claim 11 where in the contacting step comprises a first nucleic acid and a second nucleic acid, each nucleic acid having a different sequence, and complementary to or at least 90% homologous with any ten consecutive nucleotides selected from the group consisting of: 2699, 2932, 2956, 2958, 2963, 2968, and 2703.

14. A method according to claim 13 wherein the first nucleic acid and second nucleic acid are selected from the group consisting of probe sets: 2958 and 2968, and 2956 and 2958.

15. A method of detecting the presence of *Legionella* sp. in a sample suspected of containing *Legionella* comprising:

a) contacting the sample with at least one nucleic acid composition having 10 to 250 nucleotides which, under conditions that allow said nucleic acid to hybridize, hybridize preferentially with rRNA and rDNA of *Legionella*;

b) imposing hybridization conditions on the sample to form a hybridization product in the presence of *Legionella*; and

c) detecting the hybridization product as an indication of the presence of *Legionella*.

16. A method according to claim 15 wherein the nucleic acid composition which is complementary to or homologous with at least 90% of a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 2697, 2698, 2693, 2928, 2927, 2955, 2924, and 2959.

17. A method according to claim 15 wherein the contacting step comprises a first nucleic acid and a second nucleic acid, each nucleic acid having a different sequence, and complementary to or at least 90% homologous with any ten consecutive nucleotides selected from the group consisting of: 2697, 2698, 2693, 2928, 2927, 2955, 2924, and 2959.

18. A method according to claim 17 wherein the first nucleic acid and second nucleic acid are selected from the group consisting of probe sets: 2926 and 2931, and 2695 and 2696.

19. A method of detecting a member of the genus *Legionella* in a sample suspected of containing *Legionella* comprising:

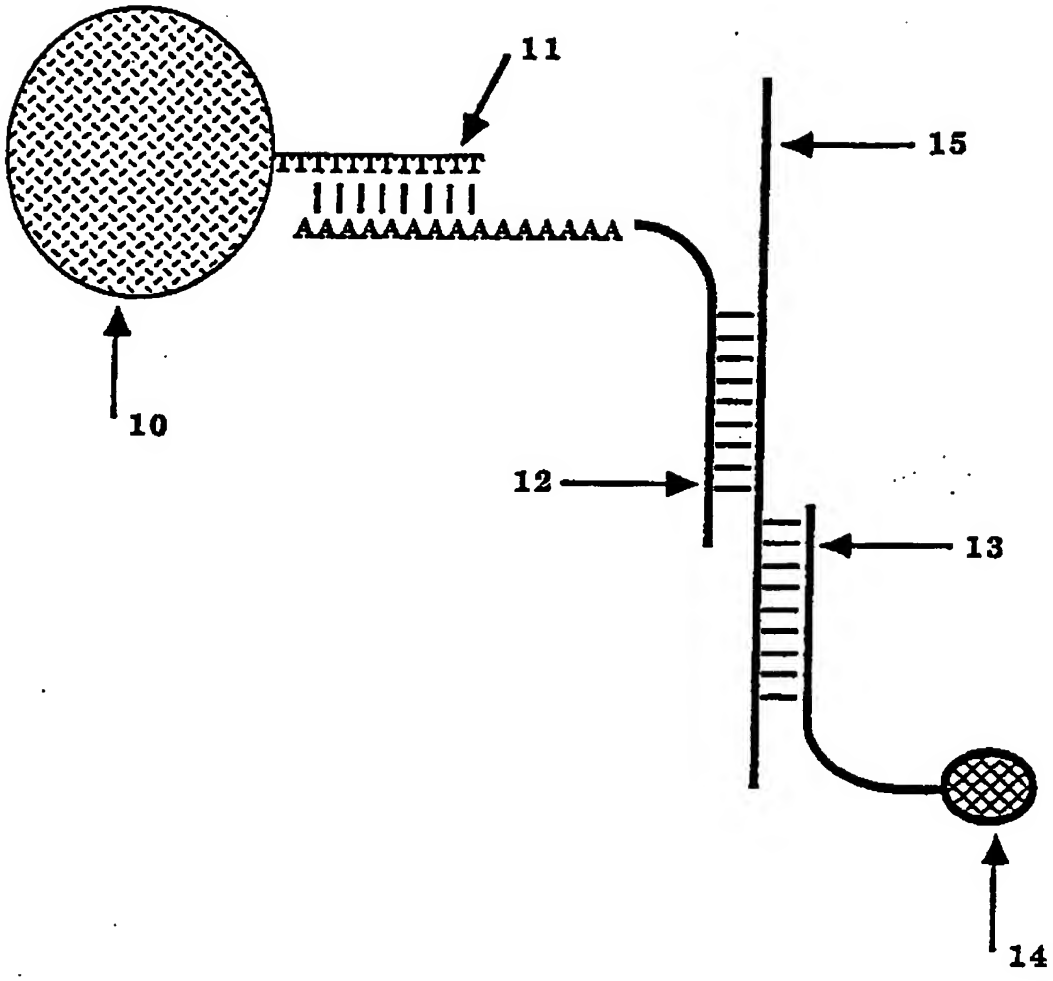
a) contacting the sample with at least one nucleic acid composition which is complementary to or at least 90% homologous with any ten consecutive nucleotides selected form the group consisting of probes 2926, 2930, 2701, 2696, and 2695;

b) imposing hybridization conditions on the sample to form a hybridization product in the presence of *Legionella*; and

c) detecting the hybridization product as an indication of the presence of *Legionella*.

20. A kit for detecting the presence of one or more of *Legionella* comprising a probe which is complementary to or at least 90% homologous with a sequence comprising any ten consecutive nucleotides selected from the group of sequences defined by probes 2701, 2703, 2704, 2705, 2679, 2690, 2698, 2695, 2696, 2693, 2708, 2699, 2924, 2926, 2930, 2932, 2956, 2958, 2963, 2968, 2928, 2957, 2927, 2929, 2954, 2955, and 2959.

FIGURE 1



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05821

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68; C07H 21/04, 21/02
US CL :435/6; 536/24.32, 23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 536/24.32, 23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS
search terms: probe, Legionella

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO, A, 88/03957 (HOGAN ET AL.) 02 June 1988, see pages 67 and 71.	1-3, 5, 7, 11, 15 --- 4, 6, 8-10, 12-14, 16-20
Y	FEMS Microbiology Letters, Vol. 65, issued 1989, Bottger, "Rapid determination of bacterial ribosomal RNA sequences by direct sequencing of enzymatically amplified DNA", pages 171-176, see entire document.	4, 6, 8-10, 12-14, 16-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 JULY 1994	Date of mailing of the international search report 29 JUL 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KENNETH R. HORLICK Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05821

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Clinical Microbiology and Infectious Disease, Vol. 95, Number 5, issued May 1991, Fain et al., "Rapid diagnosis of Legionella infection by nonisotopic *in situ* hybridization method", pages 719-724, see entire document.	4, 6, 8-10, 12-14, 16-20